PROCESSING OF HYDROXYAPATITE BY BIOMIMETIC PROCESS

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By
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CERTIFICATE

This is certified that the work contained in the project entitled "PROCESSING OF HYDROXYAPATITE BY BIOMIMETIC PROCESS" by Sakshi Jain (Roll 10608010) in partial fulfillment of the requirements of the award of Bachelor of Technology Degree in Ceramic Engineering at the National Institute of Technology, Rourkela is an authentic work carried out by her under my supervision and guidance.

To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other university / institute for the award of any Degree or Diploma.

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Sakshi Jain

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ABSTRACT

The present work deals with a novel chemical precipitation technique for synthesizing Hydroxyapatite (HAp- Ca_{10} $(PO_4)_6$ (OH) $_2$) by a Biomimetic method using calcium nitrate tetra hydrate $Ca(NO_3)_2 \cdot 4H_2O$ and diammonium hydrogen phosphate salts $(NH_4)_2HPO_4$ as precursors dissolved in synthetic body fluid (SBF) solutions at 37°C and pH of 7.4. The crystalline phase, chemical composition and crystallite size of the obtained samples, calcined at various temperatures 560°C, 750°C, 850°C, 1000°C, 1100°C and 1200°C for 2 hours, were characterized by X-ray powder diffraction (XRD). A considerable change in crystallite sizes is observed with change in calcination temperature. TG/DSC of raw powder shows that the as prepared powder has phase change in HAp at 450^0C and the total weight loss on heating was 9.45%. The specific surface area of the prepared HAp powder was measured by BET. Thus in the present work the synthesized HAp is stable till 750°C. Above this temperature, HAp first decomposes to β-TCP, followed by its conversion to α-TCP above 1200°C.

CHAPTER 1 ~ INTRODUCTION

HYDROXYAPATITE

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), also known as Hydroxylapatite and abbreviated as HAp, is an inorganic compound which is a non-toxic bioactive ceramics and whose chemical composition is similar to the composition of the bone [1]. Hydroxyapatite is the hydroxyl end member of the complex apatite group. The OH^- ion can be replaced by fluoride, chloride or carbonate, producing fluorapatite or chlorapatite. It crystallizes in the hexagonal crystal system. It has a specific gravity of 3.08 [2]. HAp as a biomaterial is given preference over other bioceramics such as bioglass or A-W glass-ceramic because of its excellent bioactivity, osteoconductivity and biocompatibility with hard tissues, skin, and muscle tissues [3].

It is widely used as a bulk material (production of bulk samples generally requires sintering at elevated temperatures, usually in excess of 1000°C), but in some cases it is used as powder or in particulate forms in biomedical applications both as a dense, sintered material such as a bone substitute material in orthopedics and dentistry and as coatings for metallic prosthesis, to improve their biological properties [1]. It is one of few materials that are classed as bioactive, meaning that it will form strong chemical bonds with surrounding bone, unlike other materials such as alumina and zirconia, which are identified as foreign materials and become encapsulated by fibrous tissue [4].

1.1 Natural Occurrence

Hydroxyapatite is a naturally occurring mineral form of calcium apatite with the formula $Ca_5(PO_4)_3(OH)$, but is usually written $Ca_{10}(PO_4)_6(OH)_2$ to denote that the crystal unit cell comprises two entities [2]. Pure hydroxyapatite powder is white. Naturally occurring apatites can, however, also have brown, yellow, or green colorations, comparable to the discolorations of dental fluorosis [2]. In the human body, HAp can be found in teeth and bones. Thus, it is commonly used as a filler to replace amputated bone or as a coating to promote bone ingrowths into prosthetic implants.

1.2 Properties

According to L. L. Hench [5], HAp has following properties:

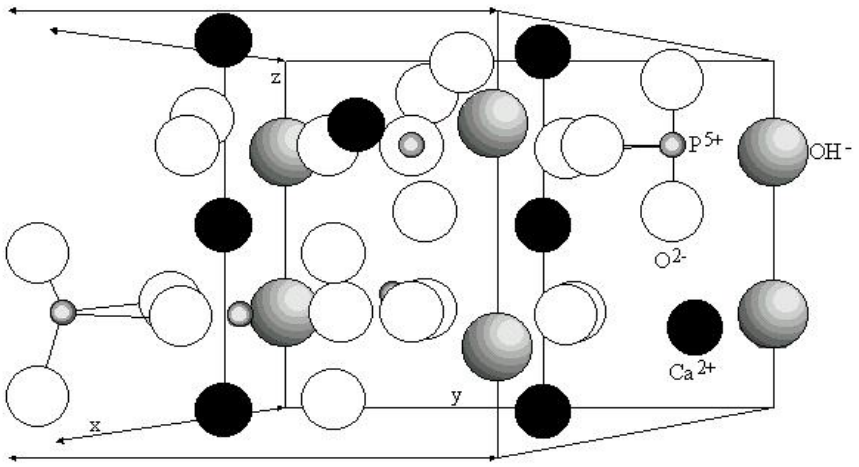
- Bioactive- The ability to integrate in bone structures and support bone ingrowth, without breaking down or dissolving.
- Biocompatible- HAp surfaces appear to be biocompatible with several cell types such as macrophages, fibroblasts, osteoclasts, osteoblasts, periodontal ligament cells. The cells cause the dissolution of the HAp ceramic crystals intra-cellularly by phagocytosis. The HAp material allows the proliferation of fibroblasts, osteoblasts and other bone cells. The cells do not seem to distinguish between HAp and bone surfaces, which indicate a significant similarity in the surface chemistry.
- Osteoconductivity – HAp allows the formation of bone on its surface by serving as scaffold or a template.

- Hydroxyapatite is a thermally unstable compound, decomposing at temperature from about 800-1200°C depending on its stoichiometry [6].
- Generally speaking dense hydroxyapatite does not have the mechanical strength to enable it to succeed in long term load bearing applications [6].

1.3 Crystal Structure

Hydroxyapatite (Ca_{10} $(PO_4)_6$ $(OH)_2$) structure consists of calcium surrounded by phosphate and hydroxyl group. It crystallizes in a hexagonal system with the following crystallographic parameters [7]: **a = 9.418 Å, c = 6.881 Å, b = 120°.**

HAp's crystallographic structure consists of a quasi-compact packing of phosphate groups, which form two types of tunnels parallel to axis C, in which the Ca(2+) ions are located. One of the apatitic structure's main characteristics is to allow a large number of substitutions, which leave the crystallographic structure unchanged [7].



Crystal Structure of Calcium Hydroxyapatite Powders Synthesized in SBF at 37°C
(Hexagonal, *P6₃/m*, a = 9.4125, c = 6.8765 Å)

Fig. 1: Crystal Structure of HAp [8]

1.4 Application of HAp [5]

- Repair of body bony defects in defect and orthopedic application.
- Immediate tooth root replacement.
- Augmentation of alveolar ridge replacements for better denture fit.
- For plasma sprayed coatings for dental and orthopedic implants.
- Application thereof in bone, connective tissue, fat tissue and muscle tissue engineering.
- Augmentation and stabilization of the jaw bone.
- Adjuvant to the placement of metal implants
- Maxillo-facial reconstruction.
- Middle ear reconstruction.
- As target materials for ion-sputtered coatings.
- Fillers in composites, or ceramics.
- As bioreactors and many more

1.5 Synthesis Routes [9]

Hydroxyapatite powders can be synthesized via numerous production routes, using a range of different reactants. Some processing techniques include

- Wet chemical methods (precipitation)
- Hydrothermal techniques
- Hydrolysis of other calcium phosphates
- Sol-gel

Of these methods the first two are the most commonly used techniques.

- **Wet Chemical Production Methods [9]**

Calcium Hydroxide and Orthophosphoric Acid

At a pH of greater than 9, orthophosphoric acid solution is added is a drop wise manner to a dilute solution/suspension of calcium hydroxide. The acid is added at a controlled rate, with stirring being maintained throughout the process. The precipitation reaction is slow. Reaction temperatures of between 25 and 90°C are common, the higher temperature producing a higher crystallinity product.

Calcium Nitrate, Diammonium Hydrogen Phosphate and Ammonium Hydroxide

Another precipitation method used for producing hydroxyapatite involves calcium nitrate, diammonium hydrogen phosphate and ammonium hydroxide. This method results in a faster production rate, with ammonium hydroxide being added to maintain a constant pH. Compared to the previous method, this approach requires washing of the precipitate to remove nitrates and ammonium hydroxide. In taking these factors into account, the production rate of these two techniques is similar.

$$10Ca(NO_3)_2.4H_2O + 6(NH_4)_2HPO_4 + 8\,NH_4OH \longrightarrow CA_{10}(PO_4)_6(OH)_2 + 20NH_4NO_3 + 20H_2O$$

- **Hydrothermal Reaction [9]**

After wet chemistry, hydrothermal techniques are the second most popular synthesis techniques for producing hydroxyapatite powders. This method involves reaction between a mixture of calcium carbonate ($CaCO_3$) and diammonium hydrogen phosphate at high temperatures and pressures such as 275°C and 12000 psi.

The resulting hydroxyapatite is carbonate substituted, but commonly well crystallized and chemically homogeneous.

1.6 Biomimetic Process

The term biomimicry and biomimetics come from the Greek words bios, meaning life, and mimesis, meaning to imitate. Other terms often used are Bionics, Bio-inspiration, and Biognosis [10-11].

Biomimetic process refers to a laboratory procedure designed to imitate a natural chemical process it is also refers to a compound that mimics a biological material in its structure or function [12]. In short, it is a human-made processes, substances, devices, or systems that imitate nature.

The art and science of designing and building Biomimetic apparatus is called Biomimetics, and is of special interest to researchers in nanotechnology, robotics, artificial intelligence (AI), the medical industry, and the military [13-15].

1.7 Advantages of Biomimetic Process [14]

Biomimetic processes mimic the bodies own bone formation and deposition processes. The Biomimetic process can be used impart bioactive characteristics onto otherwise bioinert biocompatible materials through the deposition of a bone-like apatite layer. This means that, when implanted, the human body more finds it more difficult to distinguish the implant as a foreign object, hence reducing the chance if rejection.

For implants in orthopedics or dental applications, such as hip and maxillae facial prostheses, which are typically made of titanium or cobalt alloys the apatite layer deposited on the surface of the implant will increase the rate at which bone bonds to them.

CHAPTER 2~ LITERATURE REVIEW

Tas [17] synthesized nano sized Calcium hydroxyapatite by a novel chemical precipitation technique using calcium nitrate tetrahydrate and diammonium hydrogen phosphate salts as precursors dissolved in synthetic body fluid (SBF) solutions at Biomimetic conditions of 37^{o}C and pH of 7.4. He could successfully synthesize HAp which had superior high-temperature phase stability even above 1600^{o}C.

Hench et al [5] gave basic idea about dense and porous hydroxyapatite and its different processing routes. It also discussed about the various applications of HAp as bioceramics as well as coating on metallic prosthesis.

Qiu et al. [3] synthesized nano-HAp using $Ca(NO_3)_2{\cdot}4H_2O$ and $(NH_4)_3PO_4{\cdot}3H_2O$ as precursors in the presence of polyethylene glycol (PEG). The study indicated that with changing PEG concentrations, the morphology of the powder gets affected and the crystallinity gets reduced with increasing PEG concentrations.

Kalita et al. [1] reviewed various forms of calcium phosphates and the trend of decomposition of HAp on heating. It gave the idea about the advantages of using nano HAp and nano β-TCP as bioceramics in the clinical applications.

Gross et al. [9] studied various synthesis routes of HAp. Out of many possible routes, two most commonly used synthesis routes namely Wet Chemical Precipitation and Hydrothermal reaction were discussed.

Wang et al. [11] used acicular nano-hydroxyapatite (n-HAp) to make a new biomimetic composite with polyamide (polyhexamethylene adipamide) (PA). He showed that the uniform dispersion of n-HAp in polyamide matrix was possible due to the formation of chemical binding between n-HAp and PA, such as hydrogen bonding. Interface binding and uniformly dispersion are important for enhancement of bioactivity and the mechanical property of the composite. The n-HAp/PA composite provides an opportunity to produce biomimetic materials for clinical applications.

He et al. [15] synthesized nanoflake hydroxyapatite (HAp) by a biomimetic method using $Ca(NO_3)_2.4H_2O$ and $(NH_4)_3PO_4.3H_2O$ as precursors and chondroitin sulfate (ChS) as a template. The results showed that the concentration of ChS significantly affects the morphology and growth of HA crystals. Staple-fiber-like HA crystals could be obtained at a low concentration in ChS, while flake-like HAp crystals at a high concentration of ChS. In either case, the crystallinity of synthesized HAp increased with ChS content.

Huang, et al [16] synthesized the HAp/β-TCP biphasic composites with initial Ca/P molar ratios of 1.571, 1.600 and 1.636. The results showed the phase transition from α-tricalcium phosphate (α-TCP) to β-tricalcium phosphate (β-TCP) in tricalcium phosphate/hydroxyapatite (TCP/HAp) composites. It showed that with increasing HA content in the composite, there was increase in the calcination temperature for the complete phase transition from α-TCP into β-TCP. In the process of calcination, coarsening of the particle occurred significantly.

CHAPTER 3~ EXPERIMENTAL WORK

3.1 Synthesis of Biomimetic Hydroxyapatite [17]

3.1.1 Preparation of SBF

SBF is a metastable buffer solution. Hence complete precaution to avoid variance in both preparation steps and the storage temperatures has to be maintained.

Table 1: Reagents required for preparation of SBF solution [17]

Order	Reagents	Amount (gpL)
1	NaCl (99.5%)	6.547
2	$NaHCO_3$ (99.5%)	2.268
3	KCl (99.0%)	0.373
4	$Na_2HPO_4.2H_2O$ (99.5%)	0.178
5	$MgCl_2.6H_2O$ (99.0%)	0.305
6	$CaCl_2.2H_2O$ (99.0%)	0.368
7	Na_2SO_4	0.071
8	$(CH_2OH)_3CNH_2$ (99.5%)	6.057

Steps involving preparation of SBF:

- Reagents (Table 1) were sequentially added to 700 ml of H_2O, with the restriction that a new precursor (as per Table 1) was added only after the previous addition had completely dissolved.
- A total of 40 ml of 1M HCl solution was used for pH adjustments during the preparation of 1L of SBF solution. 15 ml of this acid solution was added just before the addition of 6^{th} reagent ($CaCl_2.2H_2O$) in order to avoid turbity.
- After addition of the 8^{th} reagent ($(CH_2OH)_3\ CNH_2$), the solution temperature was raised from ambient to 37°C. It was then titrated with 1M HCl to a pH of 7.4 at 37°C.
- During titration process, it was required to dilute the solution with consecutive additions of de-ionized water in order to make the final volume to 1L.

The prepared sample of SBF solutions is to be capable of being stored at 5°C for a month without degradation [17].

3.1.2 Preparation of Ca-Hydroxyapatite powder at 37°C in SBF [17]

The raw materials used for synthesis of HAp

- Calcium nitrate tetra hydrate $Ca\ (NO_3)_2{\cdot}4H_2O$ (99**%)**
- Diammonium hydrogen phosphate $(NH_4)_2HPO_4$ (99%)
- NH_4OH solution

The procedural steps for the preparation of hydroxyapatite in SBF involve dissolution of $Ca(NO_3)_2 \cdot 4H_2O$ and $(NH_4)_2HPO_4$ in SBF solution contained in separate beakers at the start of precipitation experiments.

The amount of each precursor used in the synthesis of HAp is given as follows:

1. Amount of Ca $(NO_3)_2 \cdot 4H_2O$ taken was = 35.7149 gm
2. Amount of $(NH_4)_2HPO_4$ taken for 380 mL solution was = 7.8335 gm
3. Amount of $(NH_4)_2HPO_4$ taken for 749 mL solution was = 15.4403 gm
4. For 22 mL batch:

    4.1 Volume of NH_4OH solution taken was = 3.4920 mL

    4.2 Volume of SBF solution taken was = 6.9841 mL

5. For 55 mL batch:

    5.1 Volume of NH_4OH solution taken was = 8.7302 mL

    5.2 Volume of SBF solution taken was = 17.4603 mL

After carefully weighing each precursor, the steps indicated in flowchart Fig. were followed.

As soon as both reagents get dissolved in SBF, immediate formation of fine precipitates took place as indicated by the slight turbidity of the solution.

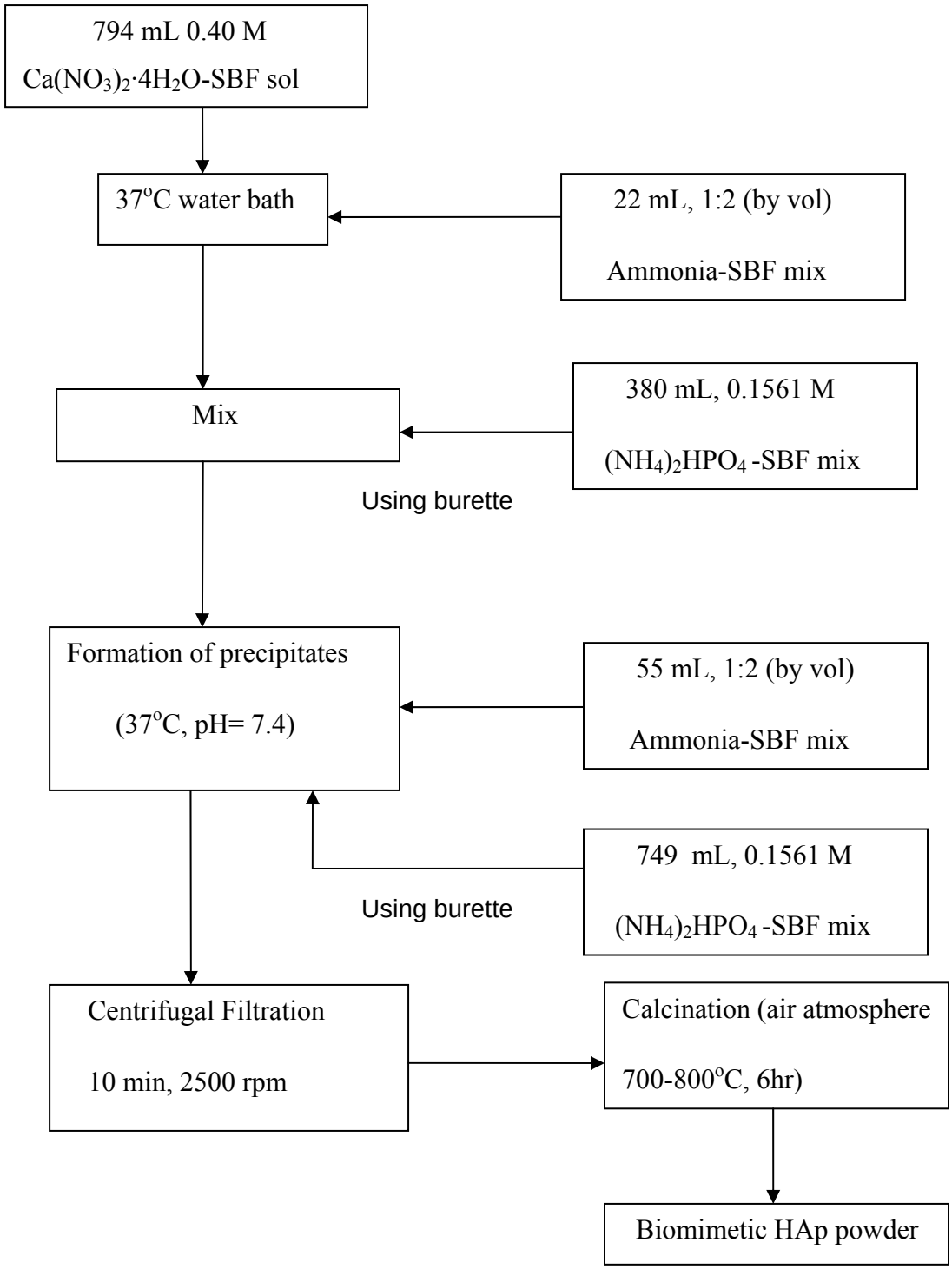


Fig 2: Process flowchart for HAp synthesis by continuous addition (of ammonia solution) technique [17].

Now, following the steps as outlined in the process flow diagram (Fig. 1), a solution was obtained.

- This solution was then filtered with filter paper of Whatman(41) with simultaneous addition of distilled water.
- After a day, the residue left behind was taken in a petridish and was kept in oven for a day.
- Next day, the dried substance was grinded in an agate-mortar till it was grinded to smooth particles.
- The obtained powder was about 15 grams.

3.2 Calcination

The as prepared HAp powder was weighed and kept in raising hearth furnace to carry out calcination at 560°C, 750°C and 850°C for 2 hours soaking time.

3.3 Compaction

For compacting the pellets, the dried powder obtained from the above procedure, was mixed with calculated amount of (PVA) binder. 3% PVA solution was added. PVA was about 2.5% of the powder. It was mixed thoroughly and was scrapped out with the help of a spatula. It was then weighed and was compacted into pellets with the help of die and punch in a hydraulic press at a load of 4 Ton for 90 seconds.

3.4 Sintering

The powder calcined at 750°C was compacted to pellet which was then sintered in a raising hearth furnace at 1000°C, 1100°C and 1200°C with 2 hours soaking at the sintering temperature. The pellets were held at 650°C for 1 hour to facilitate binder removal.

3.5 Characterization

3.5.1 DSC/ TG

Thermal decomposition and crystallization behaviour of hydroxyapatite powder was studied using DSC-TG by heating the sample at 10°C/min in argon in a thermal analyzer (Netzsch, Germany).

3.5.2 X-ray diffraction

The phase identification and the crystallite size of the HAp powder were characterized by Philips X-Ray Diffractometer (PW 1730, Holland) with nickel filtered Cu Kα radiation (λ = 1.5406 A˚) at 40 kV and 30mA. The scan range (2θ) was 20^o-80^o and scanning rate was 3^o/min.

The crystallite sizes of HAp powders were determined from the most intense peak from the X-ray diffraction data using the Scherrer formula corresponding to the full width half maxima of dominant peak:

$$D = k\lambda / \beta \cos\theta$$

Where D is the crystallite size, k = 0.9 is a correction factor to account for particle shapes, β is the full width at half maximum (FWHM) of the most intense diffraction plane, λ the wavelength of Cu target = 1.5406 A, and θ is the Bragg angle.

The volume fraction of HAp, β-TCP and α-TCP phase present was calculated by using the formula:

$$X_1 = [I_1] / [I_1 + I_2 + I_3]$$

3.6 Specific Surface Area by BET method

The most widely used technique for estimating specific surface area is the so-called BET method (Brunauer, Emmett and Teller, 1938). Under normal atmospheric pressure and at the boiling temperature of liquid nitrogen, the amount of nitrogen adsorbed in relationship with its pressure gives the specific surface area of a powder. The observations are interpreted following the model of BET Method.

Particle size of the powder sample can be determined from the specific surface area data obtained from BET method using following formula:

Particle Size = $6/(\rho * S)$

where ρ is the density of the pure HAp sample (3.16 gm/c.c) and S refers to the specific surface area of sample obtained from BET method.

3.7 Dilatometer

A dilatometer is a scientific instrument that measures volume changes caused by a physical or chemical process. Connecting rod (push rod) dilatometer, the sample which can be examined is in the furnace. A connecting rod transfers the thermal expansion to a strain gauge, which measures the shift. Since the measuring system (connecting rod) is exposed to the same temperature as the sample and thereby likewise expands, one obtains a relative value, which must be converted afterwards. [18]

3.7 Density Measurement

Density of the sintered pellets was measured by using Archimedes's principle. Distilled water was used. The dry, suspended and soaked weights of each pellet were measured to calculate the bulk density. The density was calculated by formula:

Density = {dry weight/ (soak weight – suspended weight)} *1

Relative Density = (Density obtained/ True density of Pure HAp) *100

True Density of pure HAp = 3.16 gm/c.c

CHAPTER 4 ~ RESULTS AND DISCUSSIONS

This Chapter describes the thermal and phase analysis of hydroxyapatite particles prepared through novel chemical precipitation technique using Ca $(NO_3)_2 \cdot 4H_2O$ and $(NH_4)_2HPO_4$ salts dissolved in synthetic body fluid (SBF) solutions at 37°C and pH of 7.4.

4.1 Thermal behaviour of as-prepared HAp

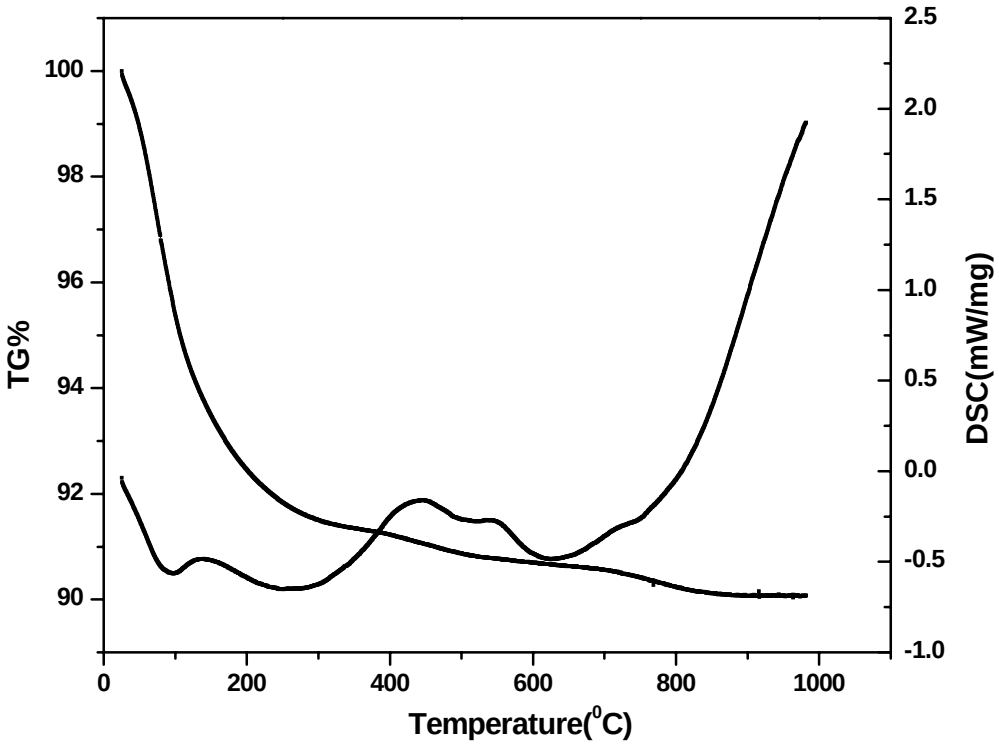


Fig. 3: DSC/TG curve of the as prepared HAp powder.

Figure 3 shows DSC/TGA data for as prepared powder of HAp. It was observed that the first two endothermic peaks at 100°C and 250°C could be accounted for evolution of absorbed water. An exothermic peak obtained at 450°C and 560°C without any associated weight loss. Thus, it corresponds to some kind of phase transformation in HAp. This was confirmed from the X-ray

pattern of as synthesized powder and powder calcined at 560^{o}C (although both the powder shows HAp peaks, there is a shift in peak position with temperature as well as appearance of new peaks (at the arrow mark positions)). Another broad exothermic peak obtained at 750^{0}C probably corresponds to β-TCP. The TGA graph shows total weight loss is 9.44%.

4.2 Phase Analysis of calcined samples:

The XRD of HAp powders prepared by novel chemical precipitation technique calcined at different temperature 560^{o}C, 750^{o}C, 850^{o}C with soaking period of 2 hr is shown in Fig.4 - 7.

4.2.1 XRD plot of raw HAp powder

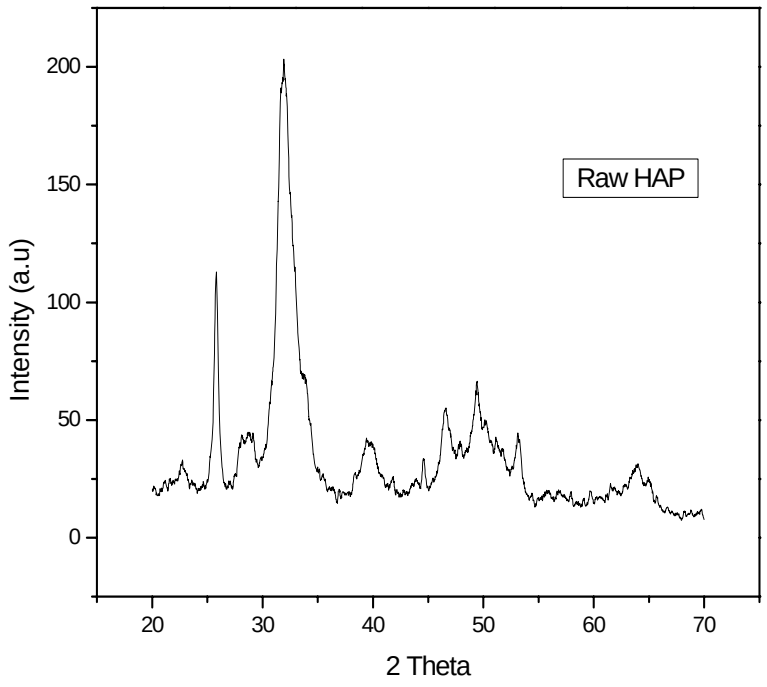


Fig. 4: XRD pattern of raw HAp powder

Fig. 4 shows XRD pattern of as prepared sample of HAp powder. The pattern in the peak positions corresponds to the HAp phase only.

4.2.2 XRD plot of HAp powder calcined at temperature 560^{o}C

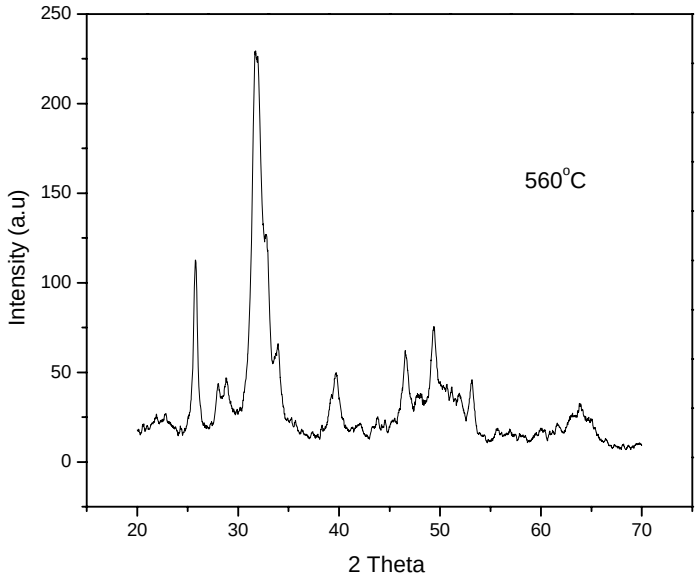


Fig. 5: XRD pattern of HAp powder calcined at temperature 560^{o}C

Fig. 5 shows XRD pattern of as prepared sample calcined at temperature 560^{o}C. This powder also shows HAp peaks like in the case of as prepared HAp sample, it is observed that there is a shift in peak position with temperature as well as appearance of new peaks. It corresponds to some kind of phase transformation in HAp.

4.2.3 XRD plot of HAp powder calcined at temperature 750°C

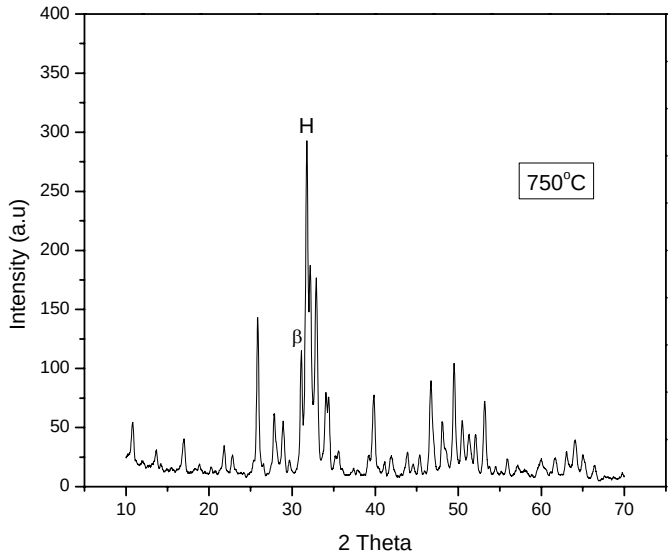


Fig. 6: XRD pattern of HAp powder calcined at temperature 750°C

4.2.4 XRD plot of HAp powder calcined at temperature 850°C

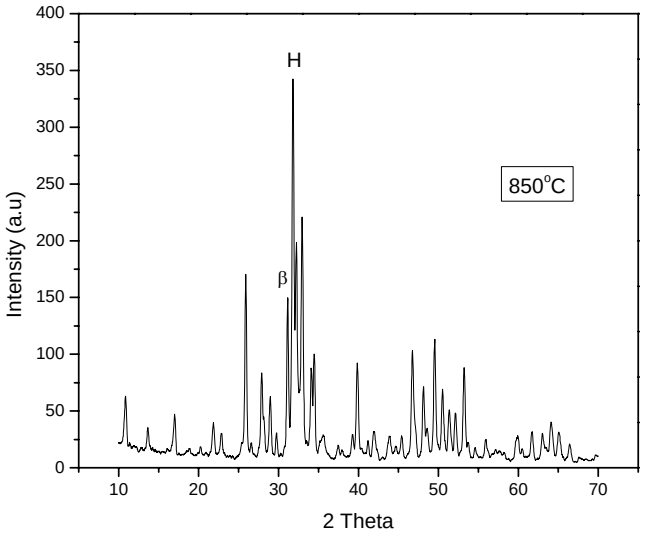


Fig. 7: XRD pattern of HAp powder calcined at temperature 850°C

Fig. 6 and Fig. 7 shows XRD patterns of as prepared sample calcined at temperature 750^{o}C and 850^{o}C respectively. These powders show HAp peaks with shift in peak position. Also it is observed that there is appearance of new peaks which corresponds to β-TCP. The volume fraction of HAp decreases and correspondingly volume fraction of β-TCP increases with increase in calcination temperature. The values are given in Table 2.

Table 2: Volume Fraction of various phases present in samples at different calcination temperature

Sl. no	Temperature(^{o}C)	Vol. Fraction of HAp (%)	Vol. Fraction of β-TCP (%)
1	750	67.94	32.68
2	850	66.15	33.85

4.3 Phase Analysis of Sintered Sample:

4.3.1 XRD plot of HAp powder sintered at temperature 1000^{o}C

Fig. 8 shows XRD pattern of as prepared sample sintered at temperature 1000^{o}C. This powder shows peaks corresponding to HAp and β-TCP.

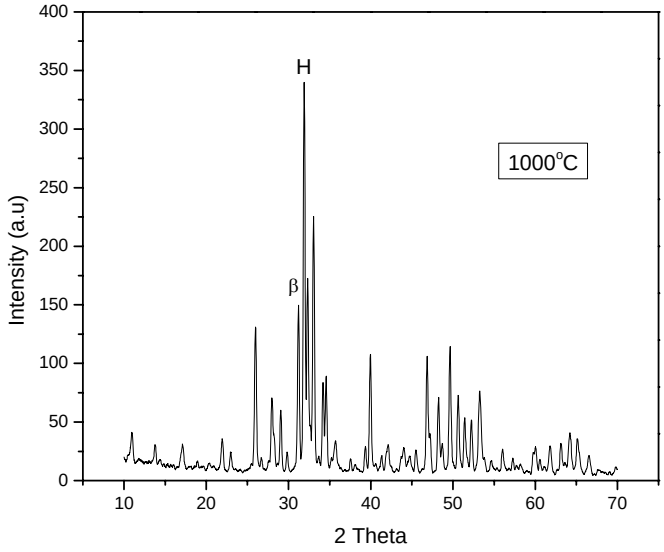


Fig. 8: XRD pattern of HAp powder sintered at temperature 1000°C

4.3.2 XRD plot of HAp powder sintered at temperature 1100°C

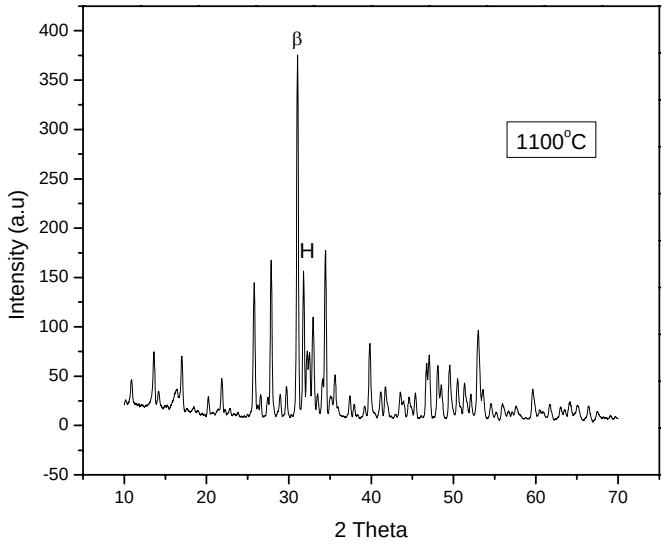


Fig. 9: XRD pattern of HAp powder sintered at temperature 1100°C

Fig. 9 shows XRD pattern of as prepared sample sintered at temperature 1100°C. This powder shows peaks corresponding to HAp and β-TCP. Although the volume fraction of HAp in this sample is lesser in value as compared to sample sintered at 1000°C.

4.3.3 XRD plot of HAp powder sintered at temperature 1200°C

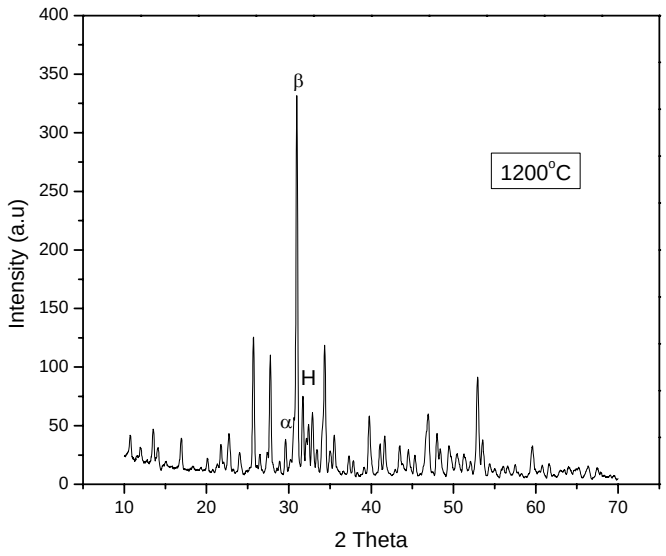


Fig. 10: XRD pattern of HAp powder sintered at temperature 1200°C

Fig. 10 XRD pattern of as prepared sample sintered at 1200°C. This powder shows peaks corresponding to HAp, β-TCP and α-TCP. The volume fraction of HAp decreases and correspondingly volume fraction of β-TCP increases with increase in calcination temperature. The values are given in Table 3.

Table 3: Volume Fraction of various phases present in sample at different sintering temperature.

Sl. no	Temperature(^{o}C)	Vol. Fraction of HAp (%)	Vol. Fraction of β-TCP (%)	Vol. Fraction of α-TCP (%)
1	1000	71.73	28.27	-
2	1100	31.15	68.85	-
3	1200	15.63	74.65	9.72

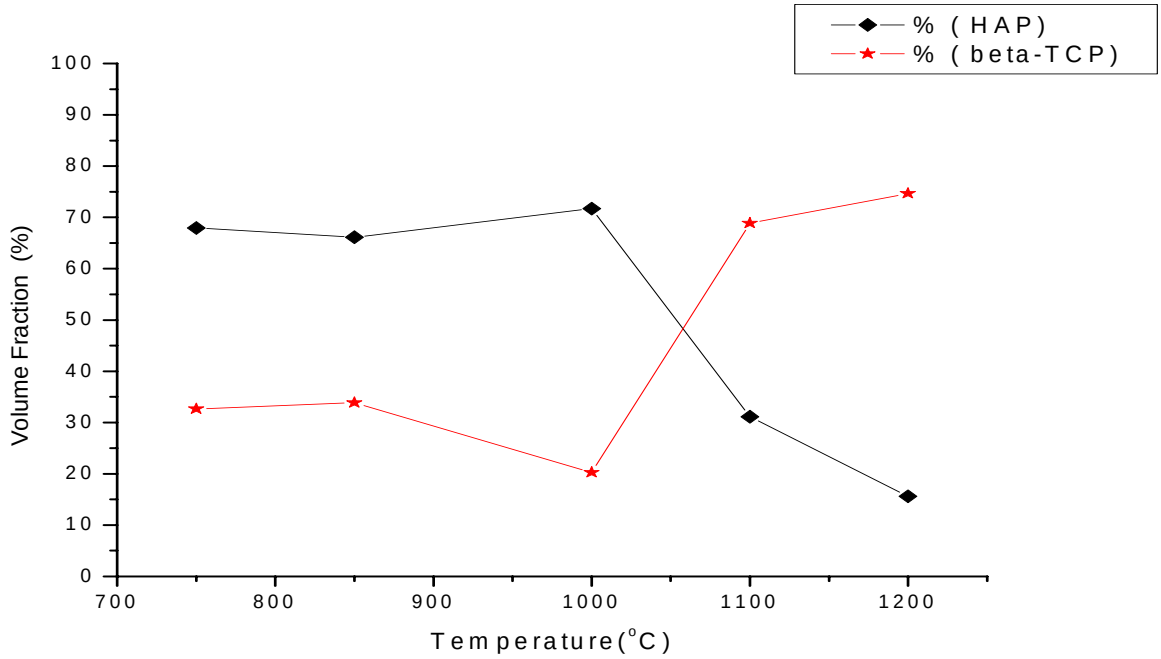


Fig 11: Comparison of volume fraction of various phases at different temperature.

The above figure shows that the volume fraction of HAp decreases with increasing temperature while the volume fraction of β-TCP it increases with increasing temperature.

4.4 Crystallite Size:

Table shows the variation of crystallite sizes along with the sintering temperature for samples.

Table 4: Crystallite sizes of the calcined samples.

Sl. NO	Calcination Temp. (^{o}C)	Crystallite Size (nm)
1	560	25.4891
2	750	45.6545
3	850	54.6399

4.5 Specific Surface Area by BET method

Following data was obtained after performing BET:

Specific Surface Area = 171.6 m^2/gm

Particle Size = 11.06 nm

4.6 Dilatometer Results

The as prepared sample of HAp powder was compacted into a rectangular pellet. It was then put in a horizontal dilatometer to study the behavior of linear shrinkage in accordance to temperature.

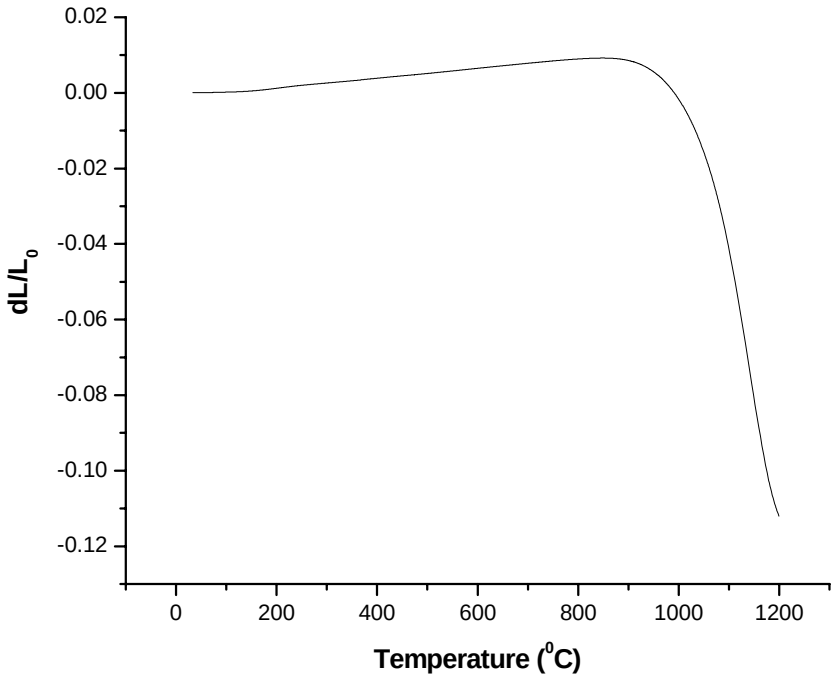


Fig. 12: Rate of change of length of sample with change in temperature.

Fig. 12 shows the rate of shrinkage of as prepared HAp sample. The total shrinkage is 12% which occurs as temperature proceeds from 750^{o}C.

4.7 Density of sintered pellets

Density was measured by Archimedes principle. It has been observed that from relative density that HAp achieves 99% at 1200^{o}C.

Table 5: Density measurement of sintered pellets

Sl. NO	Sintering Temp. (^{o}C)	Relative Density (%)	Phases Present
1	1000	63.58	HAp, β-TCP
2	1100	80.59	HAp, β-TCP
3	1200	99.30	HAp, β-TCP, α-TCP

CHAPTER 5~ CONCLUSION

Hydroxyapatite was synthesized by a Biomimetic method using calcium nitrate tetra hydrate $Ca(NO_3)_2 \cdot 4H_2O$ and diammonium hydrogen phosphate salts $(NH_4)_2HPO_4$ as precursors dissolved in synthetic body fluid (SBF) solutions at 37°C and pH of 7.4.

The HAp samples were uniaxially compacted and calcined for 2 hours at different temperatures (560, 750 and 850^oC). The powder calcined at 750^oC was then sintered for 2 hours at different temperatures (1000, 1100 and 1200^oC).

The significant findings of this work are:

1. The as prepared HAp powder contains broad peaks of HAp with crystallite size of 25.49 nm. The calcined samples show appearance of extra peaks which corresponds to decomposition of HAp to other phases.
2. All HAp peaks get clearly dissolved on calcination at 560^oC. However, at 750^oC β-TCP also appears. The powder is fine in nature.
3. The as prepared HAp sample decomposes to β-TCP above 750^oC.
4. The sample decomposes to α-TCP above 1200^oC.
5. The specific surface area was found to be 171.6 m^2/gm and the particle size was then found to be 11.06 nm.
6. For sintered samples, the maximum density was 99.3%. Although sintered sample didn't contain HAp. Almost entire phase was β-TCP and α-TCP.

CHAPTER 6 ~ SCOPE FOR FURTHER WORK

1. We can prepare HAp sample which doesn't undergo decomposition into the undesired β-TCP phase even after heating at 1600^{o}C.
2. IR spectroscopy should be carried out.
3. SEM study should be carried out.
4. Study of Bioactivity should be performed using SBF solution.

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